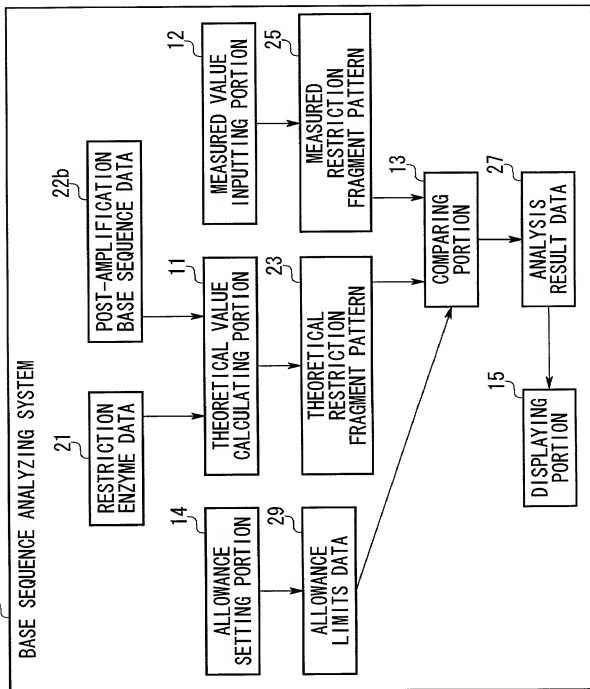


FIG. 1



## FIG. 2

LOCUS RSP16SRRZ 1490 bp rRNA BCT 04-DEC-1995  
 DEFINITION Rhodospirillum salexigens 16S ribosomal RNA.  
 ACCESSION M59070  
 NID g175871  
 VERSION M59070.1 GI:175871  
 KEYWORDS 16S ribosomal RNA.  
 SOURCE Rhodospirillum salexigens rRNA.  
 ORGANISM Rhodothalassium salexigens  
Bacteria; Proteobacteria; alpha subdivision; Rhodospirillaceae;  
Rhodothalassium.  
 REFERENCE 1 (bases 1 to 1490)  
 AUTHORS Woese, C. R.  
 TITLE A phylogenetic analysis of the some purple bacteria  
 JOURNAL Unpublished (1991)  
 FEATURES Location/Qualifiers  
 source 1..1490  
 /organism="Rhodothalassium salexigens"  
 /db\_xref="taxon:1086"  
 /tissue\_lib="DSM 2132"  
 rRNA 1..1490  
 /gene="16S rRNA"  
 /product="16S ribosomal RNA"  
 gene 1..1490  
 /gene="16S rRNA"  
 BASE COUNT 342 a 343 c 472 g 284 t 49 others

00900875.071001

## FIG. 3

ORIGIN

1 gctcagaacg aacgctggcg gcaggcctaa cacatgcaag tcgagcgcan nccttcgggg  
61 gtnagcggcg gacgggtgag taacgcgtgg gaacctgtctc agggctcttg gataactgct  
121 ggaaacggca gctaataccg gatacgccgt attgggaaa aaattcggcc ttggatggcg  
181 ccgcgttgga ttactagat ggtgggtaa cggcctacca tggcgacgat ccatagtcg  
241 tttagagga tgatcagcca cactgggact gagacacggc ccagactcct acgggagga  
301 gcagtgggga atcttagaca atgggggcaa ccctgatcta gccatgccgc gtgagtgatg  
361 aaggccttag ggttgtaaag ctctttcagc agggaagata atgactgtac ctgcagaaga  
421 agctccggct aactccgtgc cagcagccgc ggtaatacgg agngggcnag cgttgttcgg  
481 aattactggg cgtaaagcgc gcgtaggcgg atcggtcagt tgggggtgaa agcccgggcg  
541 tcaacctcgg aactgccctc aaaactaccg atcnagagtt cgggagaggt aagcgggaatt  
601 cccagtgtag aggtgaaatt cgtagatatt ggggaagaaca ccagtggcga agcggccta  
661 ctggaccgat actgacgctg aggtgcnaaa gcgtggggag caaacaggat tagataccct  
721 ggtagtccac gcgtaaacg atgggtgcta gatgtcgggg ctcttagagt ttcggtatcg  
781 cagctaacgc attaaagcacc ccgccngggg agtacggccg caagggtaaa actcaaagga  
841 attgacgggg gcnngcacaa gcggtggagc atgtggttta attcgaanna acgcgcagaa  
901 ccttaccagc tcttgacatc ccgggacgac ttccagagat ggatttttc acttcggtga  
961 cccggngaca ggtgctgcat ggctgtcgtc agctcgtgtc gtgagatgt

//

09900876-071001

## FIG. 4

### RESTRICTION ENZYME DATA

RESTRICTION ENZYME NUMBER	RESTRICTION ENZYME NAME	RECOGNITION SITE	RESTRICTION SITE
1	AluI	agct	2
2	HaeIII	ggcc	2
3	RsaI	gtac	2
4	SrfI	ccngg	2
5	HhaI	gcgc	2
6	BamHI	ggatcc	1
7	EcoRI	gaatt	1
8	HindIII	aagctt	1
9	PstI	ctgcag	5
10	PvuII	cagctg	3
11	Sall	gagctc	5
12	SmaI	cccgga	3
13	XbaI	tctaga	1

09900876-074004



THEORETICAL RESTRICTION FRAGMENT PATTERNS

FIG. 6

DNA NUMBER	NUMERICAL VALUE SECTION	GROUP NAME	NAME OF ORGANISM	NAME OF GENE	RESTRICTION ENZYME NUMBER	RESTRICTION FRAGMENT LENGTH VALUE
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	2	204
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	5	509
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	5	542
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	3	194
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	3	457
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	3	400
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	2	156
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	2	90
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	2	317
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	2	169
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	1	550
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	1	180
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	1	79
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	1	87
AB000278	1		Vibrio cholerae	16S rRNA; 16S ribosomal RNA	2	105
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	2	156
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	5	509
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	5	565
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	4	1074
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	3	194
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	3	234
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	3	223
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	3	423
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	2	90
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	1	175
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	2	204
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	2	317
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	2	105
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	1	236
AB038030	1		Vibrio splendens	16S rRNA; 16S ribosomal RNA	1	572



100170"92800660

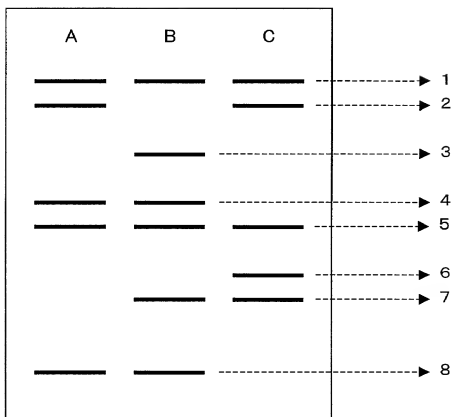
FIG. 8

MEASURED RESTRICTION FRAGMENT PATTERNS

DNA NUMBER	NUMERICAL VALUE SECTION	GROUP NAME	NAME OF ORGANISM	NAME OF GENE	RESTRICTION ENZYME NUMBER	MEASURED RESTRICTION FRAGMENT LENGTH VALUE
AP1	2	AP			1	100
AP1	2	AP			1	200
AP1	2	AP			1	300



FIG. 9



09900876.071001

	A	B	C	D
A	—	—	—	—
B	0.7	—	—	—
C	0.8	0.5	—	—
D	0.1	0.4	0.3	—

FIG. 10A

	A+C	B	D
A+C	—	—	—
B	0.6 (*1)	—	—
D	0.2 (*2)	0.4	—

\*1

$$S(B, A+C) = \frac{S(B, A) + S(B, C)}{2}$$

\*2

$$S(D, A+C) = \frac{S(D, A) + S(D, C)}{2}$$

FIG. 10B

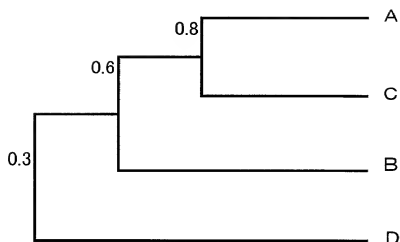
	(A+C)+B	D
(A+C)+B	—	—
D	0.3 (*3)	—

\*3

$$S(D, (A+C) + B) = \frac{S(D, A+C) + S(D, B)}{2}$$

FIG. 10C

FIG. 11



09900876-071001

FIG. 12

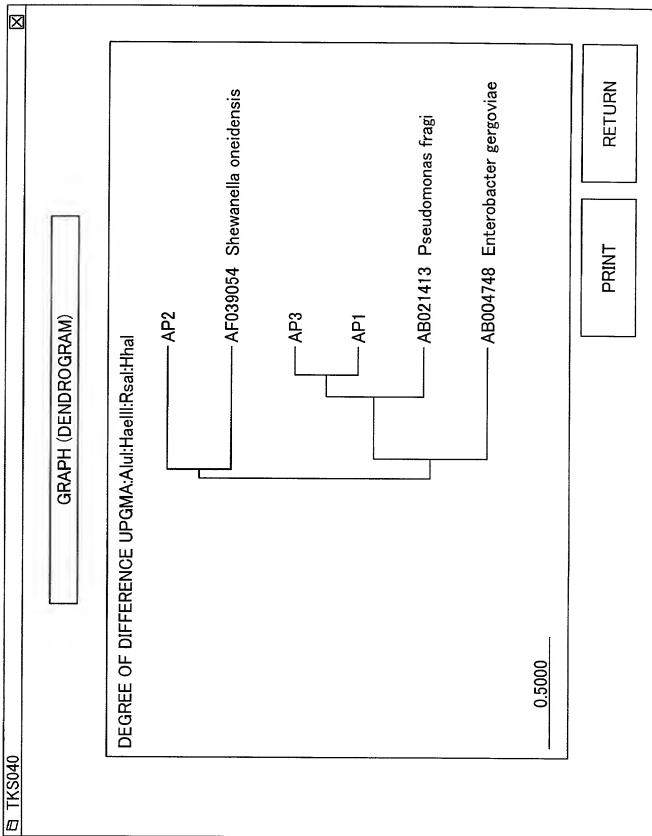
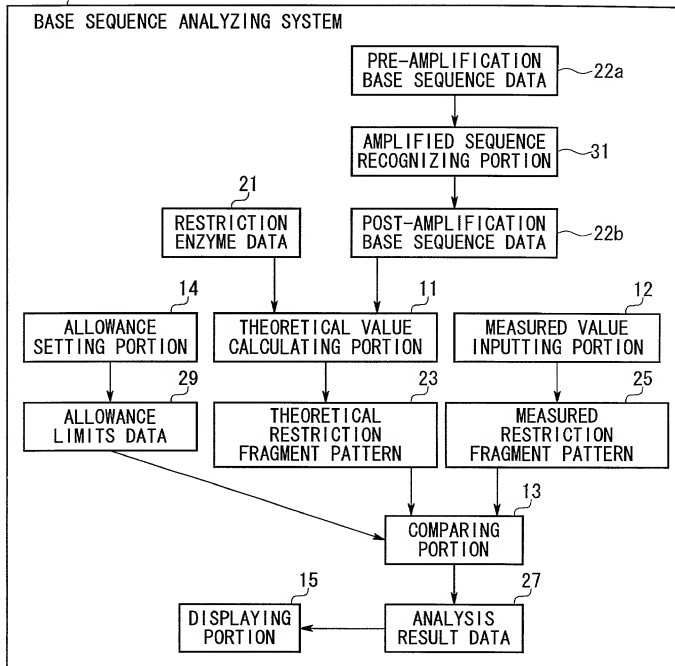


FIG. 13



09900875.071001

## FIG. 14

LOCUS RSP16SRRZ 1490 bp rRNA BCT 04-DEC-1995  
 DEFINITION Rhodospirillum salexigens 16S ribosomal RNA.  
 ACCESSION M59070  
 NID g175871  
 VERSION M59070.1 GI:175871  
 KEYWORDS 16S ribosomal RNA.  
 SOURCE Rhodospirillum salexigens rRNA.  
 ORGANISM Rhodothalassium salexigens  
 Bacteria; Proteobacteria; alpha subdivision; Rhodospirillaceae;  
 Rhodothalassium.  
 REFERENCE 1 (bases 1 to 1490)  
 AUTHORS Woese, C. R.  
 TITLE A phylogenetic analysis of the some purple bacteria  
 JOURNAL Unpublished (1991)  
 FEATURES Location/Qualifiers  
 source 1..1490  
 /organism="Rhodothalassium salexigens"  
 /db\_xref="taxon:1086"  
 /tissue\_lib="DSM 2132"  
 rRNA 1..1490  
 /gene="16S rRNA"  
 /product="16S ribosomal RNA"  
 gene 1..1490  
 /gene="16S rRNA"  
 BASE COUNT 342 a 343 c 472 g 284 t 49 others

09900876.071004

# FIG. 15

ORIGIN

```

1 ncaacatgag agtttgatcc tggctcagaa cgaacgctgg cggcaggcct aacacatgca
61 agtcgagcgc anncccttcgg gggtnagcgg cggacgggtg agtaacgcgt gggaaacctgc
121 tcagggtctct gggataactg ctggaaacgg cagctaatac cggatacgcc gtattgggaa
181 agaaattcgg ccttggatgg gcccgcttg gattagctag atgttggggg aacggcctac
241 catggcgacg atccatagct ggtttgagag gatgatcagc cacactggga ctgagacacg
301 gcccgactc ctacgggagg cagcagtggg gaattcttag caatgggggc aaccctgac
361 tagccatgcc gcgtgagtga tgaaggcctt aggggttgtaa agctctttca gcagggaaga
421 taatgactgt acctgcagaa gaagctccgg ctaactccgt gccagcagcc gcggtaatac
481 ggagngggcn agcgttggtt ggaattactg ggcgtaaagc gcgcgtaggc ggatcgggtca
541 gttgggggtg aaagcccggg gctcaacctc ggaactgcc tcaaaactac cgatcnagag
601 ttcgggagag gtaagcggaa ttccagtgat agaggtgaaa ttcgtagata ttgggaagaa
661 caccagtggc gaagcggcct tactggaccg atactgacgc tgagggtgna aagcgtgggg
721 agcaaacagg attagatacc ctggtagtcc acgccgtaaa cgatgggtgc tagatgtcgg
781 ggctcttaga gtttcgggtat cgcagctaac gcattaagca ccccgccngg ggagtacggc
841 cgcaagggtt aaactcaaa gaaattgacgg gggcnngcac aagcgtggga gcatgtggtt
901 taattcgaan naacgcgcag aaccttacc gctcttgaca tcccgggacg acttcagag
961 atggattttt tcacttcggt gaccggnga cagggtcctg atggctgtcg tcagctcgtg
1021 tcgtgagatg ttgggttaag tccncaacg agcgcaacc tcgcccttag ttgccagcat
1081 ttggttgggg actctaaggg aactgccggt gataagccgg aggaagggtg ggatgacgtc
1141 aagtcctcat ggcccttatg ggctgggcta cacacgtgct acaatggcgg tgacagaggg
1201 cagcgagcct gcgagggtga gcgaatctct aaaagccgtc tcagttcgga ttgtctctg
1261 caactcgaga gcatgaagg ggaatcgcta gtaatcgcc atcagcatgc cgcggtgaat
1321 acgttccggg gnnttgtaca caccgccgt caccacatgg gaggttggtt gaccogaaga
1381 cggtagctca acccgaagg ggggcagncg gccacggcta ggtcagcgac tggggtnnnn
1441 nngtaacaag nnnnnnnnnn nnnnnnnnnn nnnngatca cctcctttct

```

//

09900876.071001

FIG. 16

SEQUENCE OF FORWARD PRIMER, NAME (ALPHABET OR FIGURE: 5 OR LESS)		41f-1066r	
5'	<u>gctcagattgaactcgcg: 41f</u>	AF00128 AF00225 X00881	
ALLOWANCE LIMITS FOR MISMATCH		AB00120 AF00232 AF01122 D01255 D01388 D10115 D12303 X12450 X13450 X13451 X80885	
SEQUENCE OF REVERSE PRIMER, NAME (ALPHABET OR FIGURE: 5 OR LESS)			
5'	<u>acatttcacacacagcgtg: 1066r</u>		
ALLOWANCE LIMITS FOR MISMATCH		4	

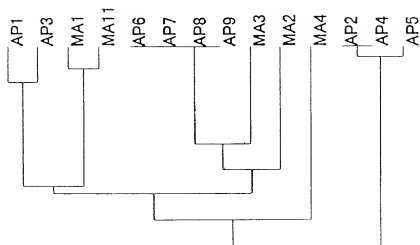


FOOT 20" 9/800660

OBLON, SPIVAK, ET AL  
DOCKET #: 210847US0X  
INV: Katsuji WATANABE, et al.  
SHEET 17 OF 29

FIG. 17

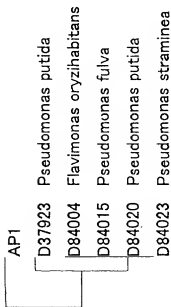
DEGREE OF DIFFERENCE UPGMA:AlutI:HaellI:RsaI:HhaI



0.5000

FIG. 18

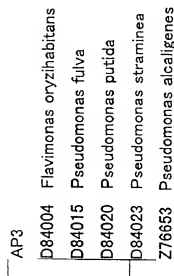
DEGREE OF DIFFERENCE UPGMA:AluI:HaeIII:RsaI:HhaI



FOOT 20" 52800660

FIG. 19

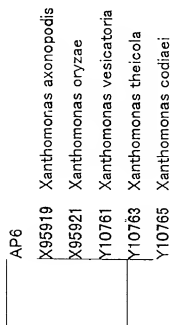
DEGREE OF DIFFERENCE UPGMA:AluI;HaeIII;RsaI;HhaI



0.5000

FIG. 20

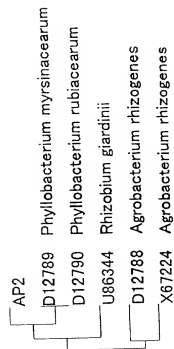
DEGREE OF DIFFERENCE UPGMA:AluI:HaellI:RsaI:HhaI



T00T20\*92800660

FIG. 21

DEGREE OF DIFFERENCE UPGMA:AluI:HaellI:RsaI:HhaI



0.5000

FIG. 22

DEGREE OF DIFFERENCE UPGMA:AluI:HaeIII:RsaI:HhaI

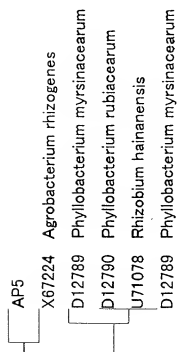
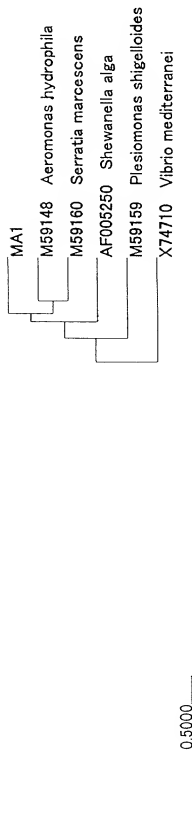


FIG. 23

DEGREE OF DIFFERENCE UPGMA:AluI:HaellI:RsaI:HhaI



TDOT20" 92800660

FIG. 24

DEGREE OF DIFFERENCE UPGMA:AluI:HaeIII:RsaI:HhaI

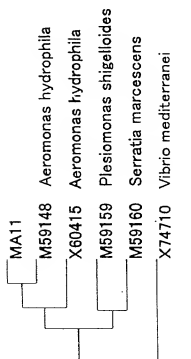
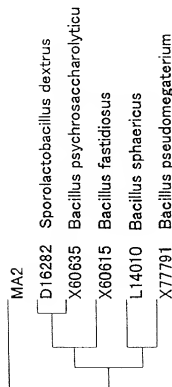




FIG. 25

DEGREE OF DIFFERENCE UPGMA-AluI:HaellI:RsaI:HhaI

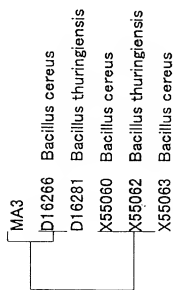


0.5000

100720-92800660

FIG. 26

DEGREE OF DIFFERENCE UPGMA:AluI:HaeIII:RsaI:HhaI



0.5000

FIG. 27

DEGREE OF DIFFERENCE UPGMA:AluI:HaeIII:RsaI:HhaI

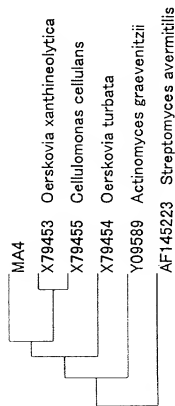
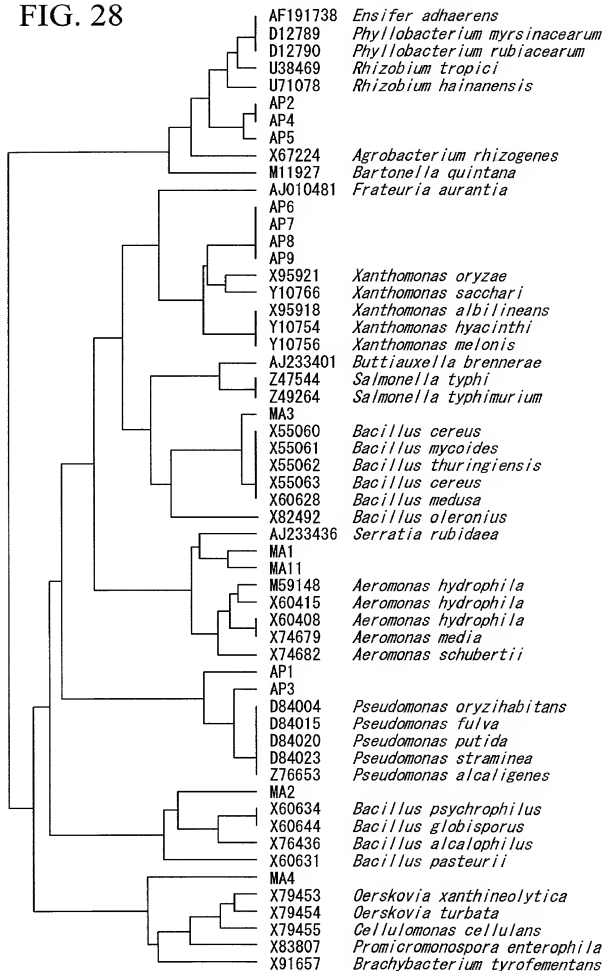


FIG. 28



09900875-071004

FIG. 29

CLASSIFICATION OF ISOLATED DENITRIFYING BACTERIA by 16S rDNA RFLP (RESTRICTION ENZYME  
HaeIII, HhaI, AluI, RsaI, SrfFI)  
AND COMPARISON OF IDENTIFICATION RESULTS BY HOMOLOG SEARCH OF BASE SEQUENCES

GROUP	R OF BAC RFLP	BASE SEQUENCE (% HOMOLOG)
I	20	<i>Enterobacteriaceae</i>
II	9	<i>Burkholderia</i> genus.
III+IV	12	<i>Ralstonia</i> genus
V	6	<i>Comamonas acidovorans</i>
VI+VIII	32	<i>Pseudomonas</i> genus
VII	20	<i>P. putida</i>
IX	8	<i>P. rhodesiae</i>
X	5	<i>P. stutzeri</i>
XI	3	<i>Acinetobacter haemolyticus</i>
XII	18	<i>Pseudomonas</i> genus
XIII	1	<i>Acivorax delafieldii</i>

*Klebsiella* genus (100%)  
*B. vietnamiensis* (96.9%, 92.6%, 93.0%, 94.8%, 93.4%), *Burkholderia* genus (100%)  
*Ralstonia* genus (92.0%, 94.5%, 94.5%), *R. paucula* (95.1%, 93.8%), *R. eutropha* (95.6%, 100%, 96.7%)  
*C. acidovorans* (98.2%, 100%)  
*P. putida* (97.7%, 99.0%, 99.2%), *P. fluorescens* (95.8%, 99.5%), *P. rhodesiae* (98.4%)  
*P. putida* (100%)  
*P. rhodesiae* (98.5%, 99.5%)  
*P. stutzeri* (98.0%, 94.6%, 92.0%)  
*A. haemolyticus* (96.1%)  
*Pseudomonas* genus (99.5%)  
*Acivorax delafieldii* (94.7%)